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Development of stable and reproducible biosensors based on electrochemical impedance spectroscopy: Three-electrode versus two-electrode setup

Luca Ianeselli [a], Gianluca Grenci [b], Carlo Callegari [c], Massimo Tormen [b], Loredana Casalis [c,*]

[a] SISSA, via Bonomea, 265, 34136 Trieste, Italy
[b] CNR-IOM, Laboratorio TASC, Area Science Park, Basovizza, Strada Statale 14 km 163.5, Trieste I-34149, Italy
[c] Elettra-Sincrotrone Trieste S.C.p.A., S.S. 14 km 163.5, In Area Science Park, Basovizza, Trieste 34149, Italy

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ABSTRACT

This work focuses on the development of electrochemical impedance biosensors based on capacitance readout, for the detection of biomolecules in small sample volumes. We performed electrochemical impedance spectroscopy (EIS) measurements of DNA hybridization in electrochemical cells with microfabricated gold electrodes. The time stability of the device was tested in two different configurations: two microelectrodes in a microfluidic channel; two microelectrodes plus a reference electrode in an electrochemical cell. Our results demonstrate that the three-electrode setup is more stable, more reproducible, and suitable for real-time measurements. In the last part of the work we perform a test study of DNA hybridization in real time, and we show that the three-electrode configuration can measure the process in situ and in real time.

1. Introduction

Recent trends in biomedicine highlight the importance of accurate and continuous monitoring of disease biomarkers during medical treatment (Gubala et al., 2012). Personalized medicine requires the development of new, faster, more reliable detectors for biomarker recognition, fulfilling the need for point-of-care medical diagnostic tools. Many detectors have been proposed in the last decade: they can be roughly divided into three big classes according to the readout technique employed: optical, mechanical or electrical. The first class includes the well-established ELISA assay (Tampoia et al., 2012), many fluorescence-based assays (Jun et al., 2012), surface plasmon resonance (SPR) (Boozer et al., 2004) and light scattering/absorption assays [Raman (Toma et al., 2012), FTIR (Gosselin et al., 2009), light scattering (Zhang et al., 2013)]. The second class relies on the variation of mechanical properties induced by the bio-recognition event and include, among others, micro cantilevers and resonators (Arlett et al., 2011), AFM (Bano et al., 2009; Mirmomtaz et al., 2008), and acoustic-wave sensors (Holford et al., 2012). These first two classes are very appealing in terms of limit sensitivity and are well suited to laboratory applications; however, they are generally built into large instruments, whose cost cannot easily be decreased to make them viable for systematic point-of-care diagnostics. Conversely, detectors in the third class, based on direct readout of an electrical signal, once miniaturized, could be easily integrated into portable electronics, at a fraction of the dimensions and total cost of competing instruments. Electrochemical chips integrated in electronic circuitry, combined with a microfluidic network that enables fast handling and reduces sample amount, were already proposed (Lai et al., 2012) and are one example of the *lab-on-a-chip* concept (Chen and White, 2011; Javanmard et al., 2009; Martins et al., 2011). Within this family, capacitance-readout-based detectors represent one of the most promising strategies (Holford et al., 2012). This type of measurements is classified as electrochemical impedance spectroscopy (EIS); either the capacitance at the electrode/electrolyte interface or the stray capacitance between two adjacent electrodes is measured, via the response to a small-amplitude AC voltage. Measurements at low frequency, where the electrode/electrolyte interface capacitance dominates are the most sensitive to capture the small variations occurring at the electrode/electrolyte interface induced by biorecognition events; the use of a third reference electrode is recommended. Complete electrochemical cells, consisting of working electrode (WE), counter electrode (CE) and reference electrode (RE), integrated into a microfluidic network, are yet difficult to realize, especially regarding RE miniaturization (Zhou et al., 2010). Many authors preferred to avoid the use of a RE and performed measurements of the impedance at the interface between

* Corresponding author. Tel.: +39 40 3478291.
E-mail addresses: loredana.casalis@elettra.eu, loredana.casalis@elettra.trieste.it (L. Casalis).

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electrode and electrolyte in a two-electrode layout, devoting special effort to the quality of the electrode surface. These experiments however, suffered from time instabilities and poor reproducibility (Carrara et al., 2009a; Mirsky et al., 1997). To solve the time drift problem, many groups focused on improving the quality of the electrode functionalization layer. Yet Mirsky et al. (1997) studied the effect of length in alkylthiols self assembled monolayers (SAMs) on the capacitance signal: electrodes passivated with long thiols (15–16 methylene groups) were more stable in time than those passivated with short thiols (up to 10 methylene groups), which are known to be less densely packed. More recently, Carrara et al. (2009a,b) performed atomic force microscopy (AFM) morphology measurements to address the short-time stability of the capacitance signal for a two-electrodes setup as a function of the surface functionalization. Although the authors claim that for more ordered SAMs no variations of the capacitance were observed within 10 min of the start of a measurement, they also state that a conditioning time of 24 h was always necessary in order to obtain reproducible, stable measurements. Such a long waiting time is incompatible with a real-time point of care diagnostic tool.

In the present experimental work we implemented and compared different biosensing platforms based on electrochemical impedance readout with the ultimate goal of performing label-free, real-time measurements of clinically relevant biomarkers. We functionalized microfabricated gold electrodes, and carried out capacitance measurements at low frequency, to highlight the small interface capacitance difference at the electrode/electrolyte interface upon binding of relevant biomolecules. We addressed the issue of time stability and reproducibility of the sensor for two different configurations: a two-microelectrodes, microfluidic device and a three-electrode electrochemical cell. In the latter, the working electrode and the counter electrode are in the micrometer scale while the reference electrode is a classical mm-sized Ag/AgCl electrode. We applied both configurations to the study of the hybridization of ssDNA SAMs on gold surfaces. The two-electrode setup turned out to suffer from a strong drift of the signal, which may take many hours and makes any use of the device as a biomolecular detector problematic. The three-electrode setup, on the contrary, showed a very good stability in time and is, for this reason, a good candidate for further development; in a preliminary test, it enabled us to measure, in real time, the hybridization kinetics of a ssDNA SAM.

2. Materials and methods

2.1. Device fabrication

Devices were fabricated using optical lithography techniques. Electrodes were first patterned on clean microscope slides using S1818 (Microposit S1800 Series Photo-Resist) as an optical positive resist. The slides were then metalized in an e-beam evaporator, with a 50-nm Ti layer followed by a 50-nm Au layer, then kept in an acetone bath overnight in order to perform the lift off process. Three-electrode devices required an additional step: after the lift-off process the electrodes were coated again with an insulating layer of S1818, shaped to expose only the circular part of the working electrode and the counter electrode. The circular working electrode exposed to the solution, the arc of the counter electrode, and the profile of the S1818 layer, which covers the rest of the metal electrodes, are visible in Fig. 1b.

The microchannels (height: 67 μm; width: 100 μm; length: 3 cm) were fabricated by pouring PDMS (Sylgard 184 Dow Corning) over a mold of SU8 100 (Nano^{TM} SU8 Microchem), then curing it at 100 °C on a hot plate for 10 min. PEEK inlet and outlet tubes (1/16 in. outer diameter) at the two ends of each channel connect it to the pump system. In order to avoid contamination a new set of electrodes and a new PDMS channel were produced for every experiment.

2.2. Differential capacitance, C_d

In this work we focus our attention on the measurement of the differential capacitance C_d at the electrode electrolyte interface. C_d is defined as:

$$C_d = \frac{\partial \sigma_M}{\partial \varphi}. \quad (1)$$

where σ_M is the charge density on the metal electrode and φ the potential difference between electrode and solution. In other words C_d measures the charge density change at the metal surface for a small variation of the applied potential. In the case of a bio-functionalized metal electrode immersed in a saline solution, C_d can be modeled with two capacitances in series (Guiducci et al., 2002): the capacitance due to the absorbed layer of molecules (C_{mol} in Fig. 2) and the one due to the ions in solution, part of the double layer capacitance (C_{dl} in Fig. 2). Since

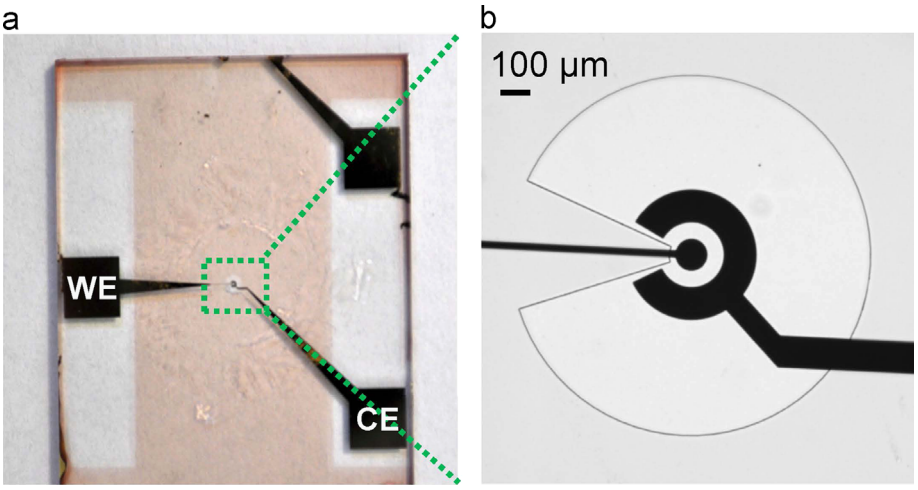

Fig. 1. (a) Picture of the microfabricated WE and CE for the three-electrode setup. The gold electrodes were fabricated on half a microscope slide and appear black in the image. The patterned insulation layer (S1818; thickness: 2.5 μm) appears pink. (b) Zoom-in of the central part of the picture. The diameter of the WE in contact with the solution is 100 μm. The patterned resist used to electrically insulate the electrodes is clearly recognizable as the darker-gray outer area of the image.